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In vivo and *in vitro* Inhibition of the Metabolism of *N*-Alkyl-substituted Amphetamines in Rat by Ferrocenylisopropylamine

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1. Ferrocenylisopropylamine (FIPA) inhibits the elimination of amphetamines in rat. The half-life of isopropylamphetamine was increased from approx. 30 to 85–100 min after administration of FIPA.

2. With isolated, perfused, rat liver, the half-lives of isopropylamphetamine, biamphetamine and benzylamphetamine were increased from 5–20 min to about 200 min by equimolar amounts of FIPA, indicating that the prolonging effect of FIPA is due to interference at the metabolic level.

3. Experiments with hepatic microsomal suspensions demonstrated that FIPA competitively inhibits the oxidative *N*-dealkylation of isopropylamphetamine; the K_I of FIPA is $4{\cdot}1 \times 10^{-6}$ M.

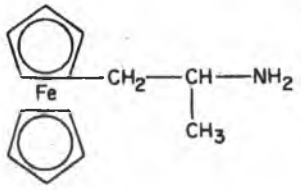
4. Binding of isopropylamphetamine and FIPA to cytochrome P-450 was studied using hepatic microsomes of phenobarbital-treated rats. Isopropylamphetamine caused a type I, and FIPA a type II difference spectrum; FIPA showed a much higher binding affinity ($K_s = 1{\cdot}24 \times 10^{-6}$ M) than isopropylamphetamine ($K_s = 0{\cdot}96 \times 10^{-3}$ M). FIPA acts as a modifier of the spectral changes induced by isopropylamphetamine.

5. Results suggest that the competitive inhibition of the *N*-dealkylation of *N*-alkylamphetamines, and thus the prolonging of their action, by FIPA is related to competition for binding to cytochrome P–450.

Introduction

Amphetamines are metabolized to varying degrees, both in animals and man, via the following pathways: *p*-hydroxylation, deamination and *N*-dealkylation. The contribution of each individual pathway to the total metabolism varies, dependent on species and on the particular amphetamine derivative. In man, for instance, amphetamines are slowly hydroxylated (Dring, Smith & Williams, 1970), whereas in rats and guinea-pigs, this reaction occurs at a much faster rate. With a single species, differences in the degree of dealkylation and deamination occur dependent on the *N*-alkyl substituent and the stereoisomer (Vree, Muskens & Van Rossum, 1971 a; Vree *et al.*, 1971 b; Beckett, Brookes & Shenoy, 1969; Gunne, 1967). For example, scission of the C–H bond, in the α-*C*-oxidation mechanism, has been shown to be the rate-limiting step for dextroisomers only

(Vree *et al.*, 1971 a, b ; Parli, Wang & McMahon, 1971), whereas *N*-oxide formation may be a determinant step with the laevoisomers.



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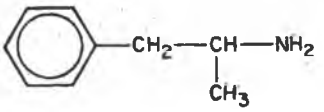

(II)

Research in this laboratory is concerned with the metabolism of amphetamines (Vree, 1973), it therefore was of interest to extend these studies to ferrocenylisopropylamine (FIPA) (I), a non-benzenoid aromatic analogue of amphetamine (II). FIPA has been reported to have little activity on the central nervous system (Loev & Flores, 1961). Current theory concerning pharmacological receptors postulates that agonist molecules must satisfy certain stereoelectronic prerequisites in order to interact effectively with a particular receptor (Ariëns, 1971). Ferrocene, although aromatic is non-benzenoid and presents a considerable physicochemical and stereochemical departure from benzene because of its cylindrical character. It might therefore be expected not to elicit a pharmacological response similar to that of amphetamine. Drug-metabolizing enzymes, on the other hand, have less structural specificity than pharmacological receptors, and are able to metabolize a variety of substrates. In these respects FIPA presented a unique compound for study. Although FIPA is an analogue of a potent pharmacologically-active agent, it is non-physiological as it contains an iron atom sandwiched between two cyclopentadiene rings, a structural arrangement which results in a considerable difference in bulk between FIPA and amphetamine.

In the present investigation, the influence of FIPA on the metabolism (*N*-dealkylation) of certain amphetamine derivatives was studied. Demonstration of competitive inhibition of the metabolism of amphetamine derivatives by FIPA would indicate that this compound had an affinity for the enzyme system concerned and might present substantial sterical hindrance to the approach of other substrates. The effects of (±)FIPA on the metabolism of (+)isopropylamphetamine and (++)biamphetamine were therefore studied. These compounds were chosen because of their short metabolic half-lives (isopropylamphetamine, 20 min ; biamphetamine, 10 min) compared to amphetamine (200 min) (Vree *et al.*, 1971 b), as within the limits of experimental procedure, it is easier to demonstrate inhibition of metabolism (increase in $t_{0.5}$) with compounds that normally have a short half-life.

Studies were carried out *in vivo* in rats, and *in vitro* with isolated perfused rat liver and hepatic microsomal suspensions. Cytochrome P-450, the terminal oxidase in the oxidative transformation of drugs, is generally believed to be the site for substrate interaction (Schenkman, 1970). Studies concerning the binding to this enzyme system, were, therefore, included.

Materials and methods

Animals

Adult male rats weighing 200–260 g were used. Phenobarbital-treated animals received 75 mg/kg sodium phenobarbital in saline intraperitoneally daily for 5 days.

In the *in vivo* experiments rats were injected intravenously with soln. of (+)isopropylamphetamine HCl and (±)ferrocenylisopropylamine HBr in saline. Blood samples (0·5 ml) were taken at regular intervals from the orbital plexus.

Isolated perfused rat liver experiments

Livers (9–14 g) were surgically removed from rats and perfused with Schimassek (1962) soln. as modified by Alvarez (1971). The liver and oxygenated (95% O_2, 5% CO_2) perfusate were maintained in a humidified chamber at 37°. Preparation of perfusion fluid was as follows: 100 ml human blood was centrifuged, the plasma removed by suction, and the remaining cells washed three times with Schimassek soln. The washed erythrocytes (10 ml) were mixed with 3 g bovine albumin, 0·150 g glucose and enough Schimassek soln. to make 100 ml. This prep. of artificial blood was carried out under aseptic conditions. One litre of Schimassek soln. contained the following salts: NaCl 137 mM; KCl 2·68 mM; $CaCl_2$ 1·80 mM; $MgCl_2$ 0·49 mM; $NaHCO_3$ 11·9 mM; $NaH_2PO_4 . H_2O$ 0·67 mM; it was sterilized by filtration.

The isolated liver was perfused with 100 ml perfusion fluid at a rate of 12 ml/min (Cardiac Suction Unit pump, type 913, New Electronic Products Ltd, London). The perfused isolated liver prep. was allowed to equilibrate for 1 h prior to an experiment. Liver function was monitored by the vol. of bile production. Test compounds were dissolved in perfusion fluid, and, at regular time intervals, 0·5 ml samples perfusion fluid were treated as indicated in the g.l.c. analysis.

Preparation of microsomes

Rats starved overnight, to remove liver glycogen, were killed by decapitation under light ether anaesthesia. The livers were rapidly excised, portions were weighed, finely minced and transferred into 9 vol. ice-cold 0·25 M-sucrose. Homogenates were made using a Teflon-glass Potter–Elvehjem type homogenizer. After sedimentation of nuclei and cell debris by centrifugation at 500 *g*, the supernatant was centrifuged at 18 000 *g* for 10 min; the microsomal fraction was isolated by centrifugation of the 18 000 *g* supernatant at 105 000 *g* for 60 min. The microsomal pellet was washed once to remove contaminating haemoglobin and carefully re-suspended in isotonic KCl soln. (0·15 M), containing 0·05 M-Tris-HCl (pH 7·5).

Determination of microsomal dealkylation

The microsomal suspension was used as the enzyme source for the measurement of *N*-dealkylation of (+)isopropylamphetamine *in vitro*. Incubations were carried out at 37°, under air, as described earlier (Henderson & Kersten, 1970). Incubation media contained: 5×10^{-2} M Tris-HCl (pH 7·5); 8×10^{-4} M-$MgCl_2$; 8×10^{-6} M-$MnCl_2$; 5×10^{-3} M-sodium isocitrate; isocitric dehydrogenase 20 μg/ml (Sigma type IV; capable of generating 5·5 μmol NADPH/min/mg at 37°); 13×10^{-5} M-NADP; and (+)isopropylamphetamine as indicated. The mixture was preincubated at 37° for 10 min, to ensure reduction of all NADP. The reaction was started by addition of the microsomal suspension, equivalent to 4 mg protein per ml. After incubation for 30 min, the reaction medium was made alkaline with KOH and extracted with ether. The amount of amphetamine

formed was determined by g.l.c. Blanks containing all reaction components including heat-denatured enzyme were similarly treated to detect non-enzymic formation of metabolites.

Microsomal difference spectra

For the measurement of difference-spectra the above microsomal suspension was diluted with 0·15 M-KCl–0·05 M-Tris-HCl (pH 7·5) to a concn. of about 1 mg microsomal protein per ml and was divided between two cuvettes. The substrate dissolved in 0·15 M-KCl–Tris-HCl (pH 7·5) was added to the sample cuvette, and an equal vol. of buffer was added to the reference cuvette. At varying substrate concn., difference-spectra were recorded with a Beckman DB–G spectrophotometer. When FIPA was used as a modifier of the spectral changes (Leibman, Hildebrandt & Estabrook, 1969) induced by (+)isopropylamphetamine, it was added to the microsomal suspension in both cuvettes.

All spectra were measured at room temp. in the absence of NADPH. Microsomal protein concn. were determined by the method of Lowry, *et al.* (1951).

Gas chromatographic analysis

An internal standard, methylbenzylamine (10 μg) was added to 0·5 ml samples of perfusion fluid, and the resulting soln. made alkaline with 2 ml 20% KOH, extracted with 10 ml freshly distilled ether, and the ether extract conc. to 100 μl in a stream of dry air. The conc. extract (5 μl) was injected into the g.l.c. and the concn. of the test compounds and their metabolites calc. relative to the internal standard. The g.l.c. instrument was a Hewlett Packard Model 402, with flame ionization detector, and a Mosely recorder model 7127 (1 mV full scale deflection). The operating conditions were as follows: 20% Apiezon 5% KOH on Gaschrom Q60–80 mesh, or 3% Carbowax 2% KOH on Gaschrom Q60–80 mesh, in a column 1·80 m by 3·0 mm diam.; carrier gas flow rate: N_2, 20 ml per min; H_2, 30 ml per min; air, 150 ml per min; temp.: oven 160°; flash heater 200°; detector 200°.

Synthesis

All compounds were prepared according to previously published methods (Loev and Flores, 1961; Vree, *et al.*, 1971 b) and their purity established by m.p., i.r., u.v., n.m.r. and mass spectral data.

Results

A. *In vivo metabolism in rats*

The results of these experiments are presented in Table 1. Rats rapidly eliminate isopropylamphetamine and the half-life for this compound is approx. 30 min. FIPA had a half-life of approx. 200 min (120–300 min), which is similar to that of amphetamine itself. Administration of (±)FIPA, in suitable concn., together with (+)isopropylamphetamine caused a change in the half-life of the latter to 85–100 min (Table 1, Fig. 1). In order for this effect to become manifest, the blood concn. of FIPA had to be of the same order as isopropylamphetamine. This was due to the large difference in the fictive volume of distribution

Table 1. Elimination of (+)isopropylamphetamine and ferrocenylisopropylamine by the rat

The fictive volume, $V_f = D/C_0$ = dose/blood concn. at $t = 0$

$$\frac{V_f \text{ ferrocenylisopropylamine}}{V_f \text{ (+)isopropylamphetamine}} = 5{\cdot}8.$$

Rat	Compound	Dose (μ mol)	$t_{0{\cdot}5}$ (min)	V_f (ml)
A	(+)Isopropylamphetamine	5·95	25	503
A	(+)Isopropylamphetamine	1·48	30	580
B	(+)Isopropylamphetamine	4·25	30	576
A	(±)Ferrocenylisopropylamine	39·2	180	4080
A	(±)Ferrocenylisopropylamine	13·9	120	2880
B	(±)Ferrocenylisopropylamine	3·87	300	2770
A	(+)Isopropylamphetamine plus ferrocenylisopropylamine	3·96 13·9	85 130	775
B	(+)Isopropylamphetamine plus ferrocenylisopropylamine	4·25 14·9	100 120	576

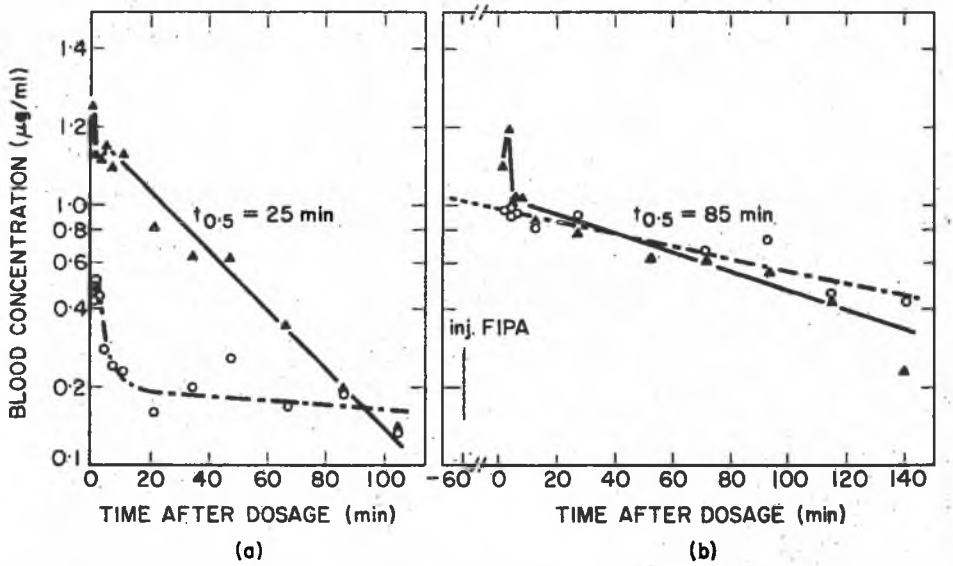

Fig. 1 *Blood concentration of* (+)*isopropylamphetamine and* (+)*ferrocenylisopropylamine after intravenous dosage to rats.*

Rat A was injected intravenously with (+)isopropylamphetamine and (±)ferrocenylisopropylamine (FIPA) and blood concn. of the compounds were estimated at different time intervals.

In the experiment shown in (*a*), (+)isopropylamphetamine (0·79 mg) and (±)ferrocenylisopropylamine (0·78 mg) were injected but the amount of FIPA was too low for inhibition. The $t_{0{\cdot}5}$ of (+)isopropylamphetamine (25 min) was the same as observed in control experiments; ▲ = isopropylamphetamine; ○ = ferrocenylisopropylamine. In (*b*) inhibition of elimination of (+)isopropylamphetamine (0·7 g) is shown after intravenous administration of a suitable dose of FIPA (3·61 mg), injected 1 h before isopropylamphetamine in order to avoid interference by the distribution phenomenon as shown in (*a*); ▲ = isopropylamphetamine ($t_{0{\cdot}5}$ = 85 min); ○ = ferrocenylisopropylamine ($t_{0{\cdot}5}$ = 130 min).

(V_f = dose/blood concn. at zero time) between the two compounds, for, as can be seen from the data in Table 1, FIPA has a V_f approx. 6 times greater than isopropylamphetamine. The prolongation of the half-life of isopropylamphetamine by FIPA indicates that the latter compound hinders the elimination of the former *in vivo*.

B. *Isolated perfused liver experiments*

The results of these experiments are presented in Tables 2 and 3. The half-life times of metabolism and the fictive volumes of distribution of a number of amphetamine derivatives are given in Table 2. Amphetamine and its *N*-methyl and *N*-ethyl derivatives have metabolic $t_{0.5}$ values of between 100 and 200 min. In contrast to this, the higher homologues, (+)isopropylamphetamine, (+)benzylamphetamine and (++)biamphetamine, have metabolic $t_{0.5}$ values of between 5 to 20 min. The *N*-alkyl amphetamines as a group are dealkylated in the isolated liver prep. to amphetamine as shown schematically below:

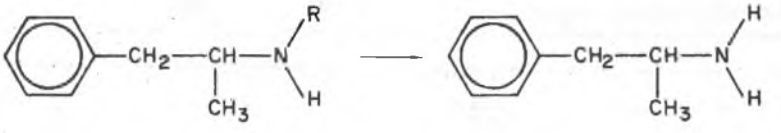

Table 2. Half-life times of metabolism and fictive volumes of distribution of some amphetamines in the isolated perfused rat liver

$$\frac{V_f \text{ ferrocenylisopropylamine}}{V_f \text{ (+)isopropylamphetamine}} = 5{\cdot}8.$$

Exp.	Compound	Dose (μ mol/liver)	$t_{0.5}$ (min)	V_f (ml)	Liver wt. (g)
4	(+)Isopropylamphetamine	29·6	20	129	12·4
21	(+)Isopropylamphetamine	40·6	20	115	14·4
28	(+)Isopropylamphetamine	27·6	20	192	11·7
13	(−)Isopropylamphetamine	36·0	43	129	14·4
14	(+)Benzylamphetamine	24·8	13	187	10·9
15	(−)Benzylamphetamine	24·0	20	175	13·3
23	(++)Biamphetamine	20·0	20	167	9·4
29	(++)Biamphetamine	18·5	5	190	9·7
30	(++)Biamphetamine	19·0	5	120	10·5
31	(++)Biamphetamine	28·2	5	125	9·3
17	(+−)Biamphetamine	20·6	27	185	9·9
19	Ferrocenylisopropylamine	18·6	400	900	10·6
20	Ferrocenylisopropylamine	26·0	200	700	7·7
21	Ferrocenylisopropylamine	28·6	120	775	14·4
23	Ferrocenylisopropylamine	28·0	200	600	9·4
31	Ferrocenylisopropylamine	24·8	130	300	9·4
28	*N*-isopropyl ferrocenylisopropylamine	25·8	50	500	11·7
30	*N*-isopropyl ferrocenylisopropylamine	16·6	40	1000	10·5

Table 3. Inhibition of metabolism of (+)isopropylamphetamine and (++)biamphetamine by ferrocenylisopropylamine in isolated perfused rat liver

Exp.	Compound	Dose (μ mol/liver)	$t_{0.5}$ (min)	V_f (ml)	Liver wt. (g)
20	(+)Isopropylamphetamine	24·7	240	145	7·7
	plus				
	ferrocenylisopropylamine	26·0	200	700	
21	(+)Isopropylamphetamine	40·6	200		14·4
	plus				
	ferrocenylisopropylamine	28·6	120	775	
28	(+)Isopropylamphetamine	27·6	200	192	11·7
	plus				
	N-isopropyl ferrocenylisopropylamine	25·8	50	500	
23	(++)Biamphetamine	20·0	160		
	plus				
	ferrocenylisopropylamine	28·0	200	600	9·4

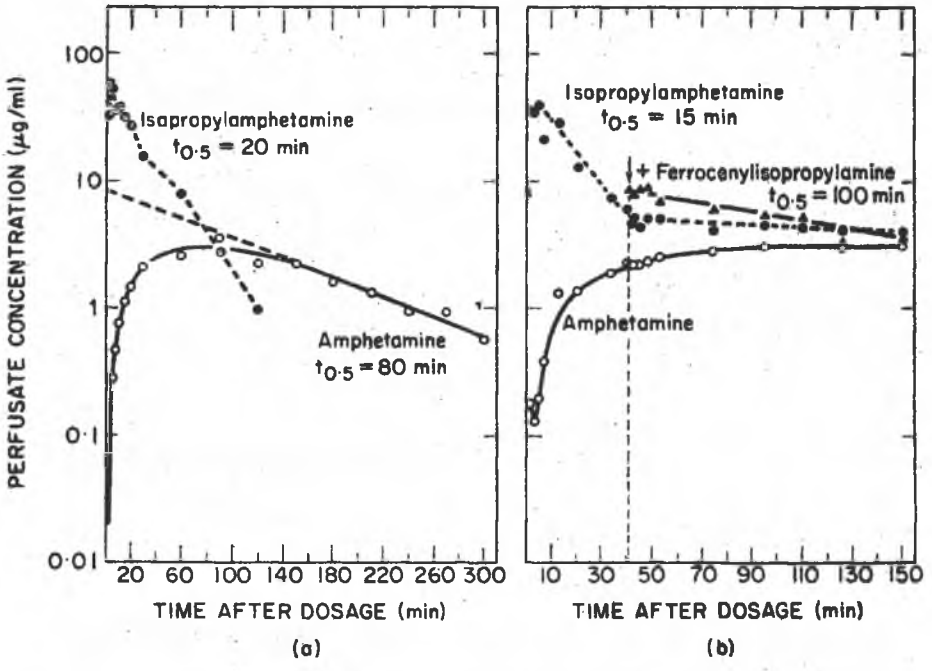

Fig. 2. *Metabolism of* (+)N-*isopropylamphetamine in the isolated perfused rat liver.*

(*a*) *Metabolic conversion of* (+)*isopropylamphetamine into amphetamine.* (+)*N*-isopropylamphetamine HCl (7·2 mg) was added to the perfusion system. (*b*) *Inhibition of metabolism of isopropylamphetamine and amphetamine.* (+)*N*-isopropylamphetamine HCl (5·25 mg) and (±)ferrocenylisopropylamine HBr (6·20 mg) and (±)ferrocenylisopropylamine HBr (6·20 mg) were added to the perfusion system. In order to elicit a clear-cut inhibition, ferrocenylisopropylamine was added about 40 min after the (+)isopropylamphetamine.

FIPA was hardly metabolized during the period of the experiment and therefore has a long metabolic $t_{0.5}$, of the order of 300 min. When FIPA and isopropylamphetamine were added in equimolar amounts to the perfused liver system the metabolic $t_{0.5}$ of the latter was changed from 20 min to approx. 200 min (Fig. 2, Tables 2 and 3) indicating a considerable inhibition of metabolism. The result of this inhibition was a fairly constant concn. of amphetamine (by dealkylation of isopropylamphetamine) over a long period of time. Similar results were obtained when FIPA was combined with benzylamphetamine and biamphetamine.

When *N*-isopropyl FIPA was added to the perfused liver prep., FIPA was detected as the metabolite, which suggests that FIPA is reversibly bound to the metabolizing enzyme system.

C. *Effect of ferrocenylisopropylamine on the microsomal* N-*dealkylation of* (+)*isopropylamphetamine*

The influence of (±)FIPA on the oxidative *N*-dealkylation of (+)isopropylamphetamine was investigated by incubating the compounds in suspensions of rat liver microsomes. The rates of enzymic conversion of (+)isopropylamphetamine were measured at constant substrate concn. with varying concn. of inhibitor.

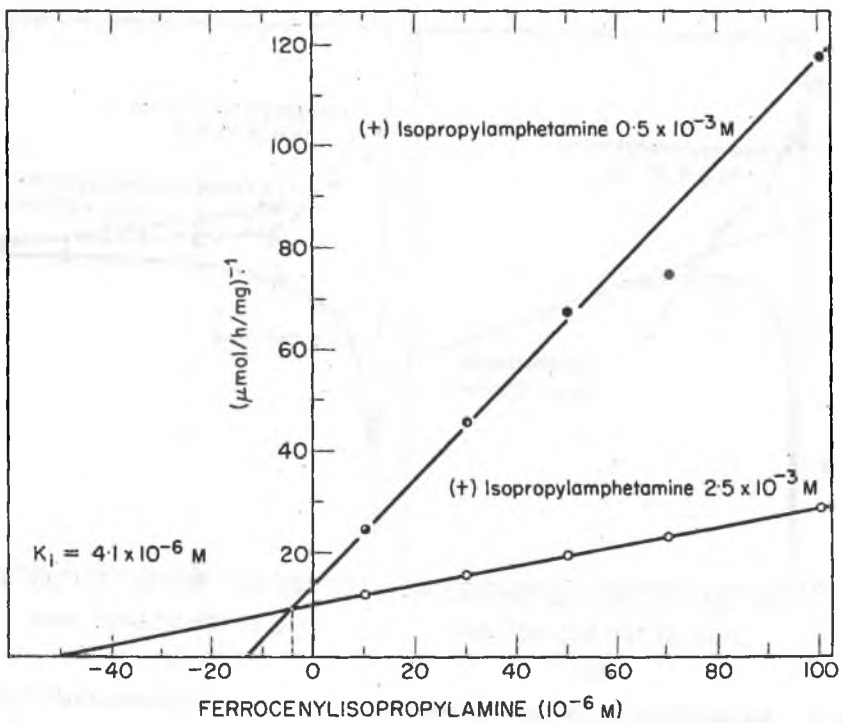

Fig. 3. *Dixon plot of the effect of ferrocenylisopropylamine on the* N-*dealkylation of* (+)*isopropylamphetamine by hepatic microsomes.*

Abscissa : concn. of ferrocenylisopropylamine ; ordinate : reciprocal rate of enzymic conversion, expressed as μmol amphetamine formed/h/mg microsomal protein. Conditions are as described in Methods ; livers of phenobarbital-treated rats were used.

Data were treated according to Dixon (1953) and the results are shown in Fig. 3. The results indicate a competitive inhibition of microsomal dealkylation by (±)FIPA. The inhibitor constant (K_i) was calculated to be $4{\cdot}1 \times 10^{-6}$ M.

D. *Interactions at the level of cytochrome P–450*

Addition of (+)isopropylamphetamine and (±)FIPA to microsomal suspensions derived from rat liver produced characteristic spectral changes. Two distinct types of difference spectra were observed with these compounds. (+)Isopropylamphetamine caused a type I spectrum, which is shown in Fig. 4; the absorbance changes at the max. (395 nm) and at the trough (425 nm) appeared to be related to the concn. of substrate. Double reciprocal plots of these data were made according to the method of Lineweaver and Burk, and an apparent dissociation constant (K_s) of $0{\cdot}96 \pm 0{\cdot}18 \times 10^{-3}$ M was obtained for (+)isopropylamphetamine. This value corresponds very well with the apparent K_m of $0{\cdot}67 \times 10^{-3}$ M, determined during the microsomal dealkylation experiments. (±)FIPA, on the other hand, gave a type II difference spectrum with a peak and a trough at 425 and 395 nm, respectively (see Fig. 5); an apparent K_s of $1{\cdot}24 \pm 0{\cdot}15 \times 10^{-6}$ M was calculated from a Lineweaver–Burk plot (Fig. 6). The value of this spectral dissociation constant is of the same order of magnitude as the K_i measured for (±)FIPA ($4{\cdot}1 \times 10^{-6}$ M) determined during the inhibition of microsomal dealkylation of (+)isopropylamphetamine.

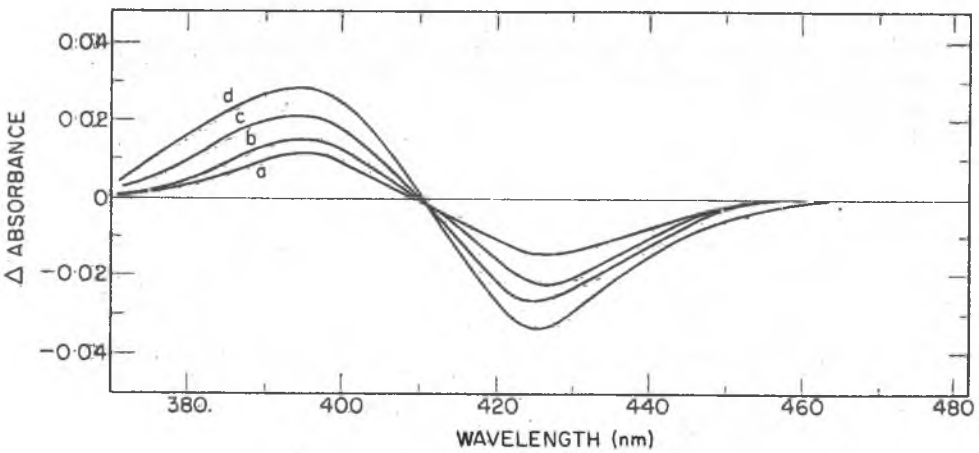

Fig. 4. *Type I spectral changes obtained on addition of (+)isopropylamphetamine, at different concentrations, to hepatic microsomes of rats pretreated with phenobarbital.*

The microsomes were diluted with Tris-KCl (pH 7·5) to a final concn. of 1 mg protein ml (+)isopropylamphetamine was added at the following concn.: (*a*) 5×10^{-4} M; (*b*) 1×10^{-3} M; (*c*) 2×10^{-3} M; and (*d*) 5×10^{-3} M.

Preliminary studies revealed that FIPA is deaminated, although slowly, to ferrocenylacetone by microsomal preparations from rat liver in the presence of NADPH. This may indicate that FIPA itself can also serve as a substrate for microsomal oxidation and probably binds to cytochrome P–450 at the active site. As mentioned previously, *N*-isopropyl-FIPA is dealkylated to FIPA. Since

deamination and *N*-dealkylation are considered to be the result of one enzymic action, α-*C*-oxidation (Becket, 1971 ; Vree, *et al.*, 1971 a), the relatively fast dealkylation of *N*-isopropyl-FIPA and the simultaneous inhibition of isopropylamphetamine (Table 3) also support the idea that these ferrocenes bind at the active site of P–450. Since the K_s can be regarded as an inverse measure of the affinity of a particular substrate for cytochrome P–450, the large difference in the spectral dissociation constants of (+)isopropylamphetamine and (±)FIPA substantiates the view that the latter compound would act as a competitive inhibitor.

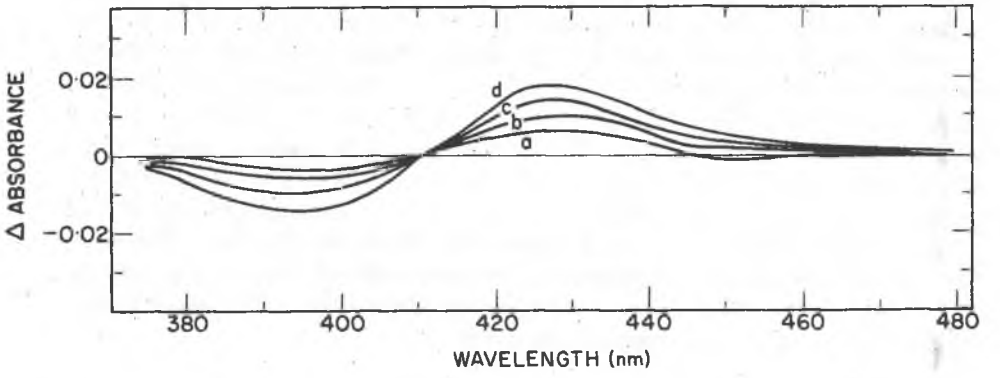

Fig. 5. *Type II spectral changes observed after addition of different concentrations of ferrocenylisopropylamine to liver microsomes of rats pretreated with phenobarbital.*

The final microsomal protein concn. was 1 mg/ml ; ferrocenylisopropylamine was added at the following concn. : (*a*) 5×10^{-7} M ; (*b*) 1×10^{-6} M ; (*c*) 3×10^{-6} M ; and (*d*) 5×10^{-6} M.

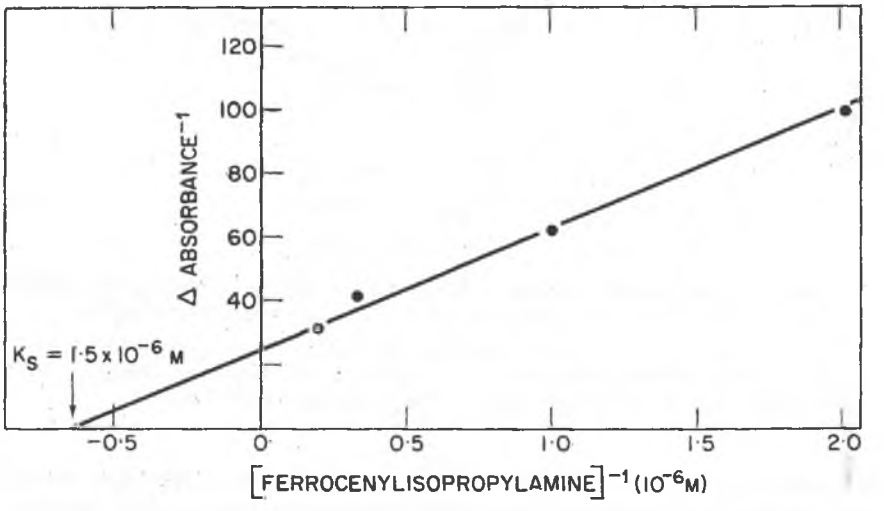

Fig. 6. *Lineweaver–Burk plot of the spectral changes induced by addition of ferrocenylisopropylamine to rat liver microsomes.*

The microsomal suspension contained 1 mg of protein/ml. The changes in absorbance were calc. at 395 and 425 nm, with respect to the base line.

The effect of (±)FIPA upon the binding of (+)isopropylamphetamine to cytochrome P–450 was also studied. In these experiments, difference spectra were recorded after the addition of increasing amounts of (+)isopropylamphetamine, in the presence of a constant concn. of (±)FIPA (the inhibitor was added to the microsomal suspensions beforehand). A double reciprocal plot was made of the sum of the absorbance changes at the peak and the trough against the concn. of (+)isopropylamphetamine (Fig. 7). It may be seen that the (±)FIPA acts as a competitive inhibitor; the K_s of (+)isopropylamphetamine was changed from $0{\cdot}83 \times 10^{-3}$ M in the control experiment, to 1·45 and $4{\cdot}15 \times 10^{-3}$ M in the presence of 1·0 and $5{\cdot}0 \times 10^{-6}$ M (±)FIPA, respectively. Analogously to the classical competitive inhibition model in enzymology, a dissociation constant of (±)FIPA (spectral K_i) can be calculated and amounts to $1{\cdot}30 \times 10^{-6}$ M on average. This value is quite similar to the K_s-value of (±)FIPA ($1{\cdot}24 \times 10^{-6}$ M).

Discussion

The data presented herein clearly demonstrate that FIPA competes for sites on the enzymes that normally metabolize amphetamine derivatives. This further indicates that the enzymes are capable of accommodating very bulky substrates; in fact, in the present study, FIPA demonstrated a 1000-fold greater affinity to the enzyme system, when compared with (+)isopropylamphetamine. It would be reasonable to assume at this point that the ferrocenyl moiety contributes substantially to this increase in binding energy.

FIPA was also a competitive inhibitor for the *N*-demethylation of aminopyrine (α-*C*-oxidation), and it remains to be determined whether or not FIPA is a general inhibitor of microsomal oxidations.

In the *in vivo* situation, the relative V_t values of FIPA and isopropylamphetamine determine the effectiveness, via distribution, of the former compound as a

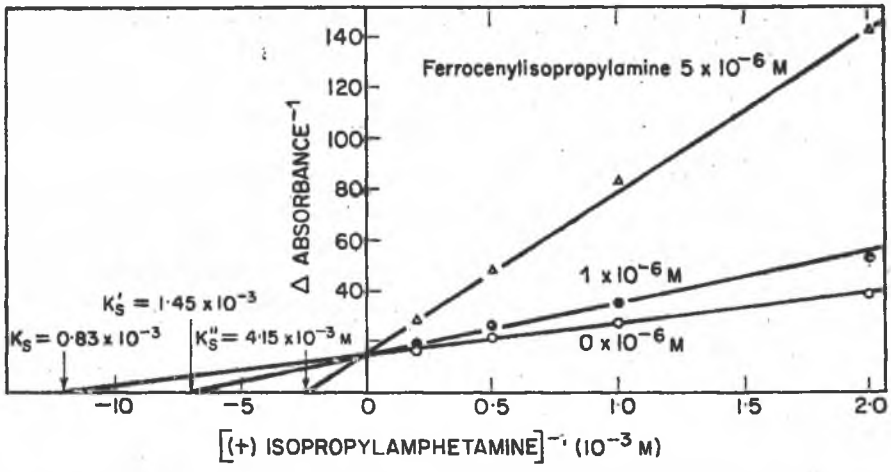

Fig. 7. *Effect of ferrocenylisopropylamine upon the spectral changes induced by addition of (+)isopropylamphetamine to rat liver microsomes (Lineweaver–Burk plot).*
Livers of phenobarbital-treated rats were used. The microsomal suspension contained 1 mg of protein/ml. Apparent K_s-values of (+)isopropylamphetamine are shifted to higher concn. by the presence of ferrocenylisopropylamine.

competitive inhibitor of liver *N*-dealkylation. The V_t ratio of FIPA/isopropylamphetamine is approximately 6, indicating that the ferrocenyl derivative is much more widely distributed throughout the body tissues. This corresponds very well with the work of Dratz, Coberly & Goldstein (1964), in which it was shown that radioactively labelled ferrocene initially concentrates in fat tissue in large quantities. However, as partition experiments in our laboratory indicate that isopropylamphetamine is at least 10 times more lipid-soluble than FIPA (see Table 4), the difference in the V_t values might be explained by selective binding.

Table 4. Apparent and true partition coefficients of amphetamine, ferrocenylisopropylamine, and derivatives

Compound	pK_a	APC_h*	APC_{chl}*	TPC_h†	TPC_{chl}†
Amphetamine	9·90	0·005	0·48	1·88	146
Isopropylamphetamine	10·14	0·21	8·09	117	4460
Ferrocenylisopropylamine	9·70	0·075	2·33	15·0	466
N-Isopropylferrocenyl-isopropylamine	9·85	4·00	99·0	1130	28 000

*APC = apparent partition coefficient.
†TCP = true partition coefficient;
h = *n*-heptane–water (Theorell buffer, pH 7·4);
chl = chloroform–water (Theorell buffer, pH 7·4).

Increase of the FIPA/isopropylamphetamine molar ratio, as in our *in vivo* experiments, results in a proportionate increase in the initial FIPA concentration obtained in the liver, which permits the compound to exert a measurable inhibition of metabolism. Dratz, *et al.* (1964) have also shown that after rapidly attaining high concentrations in fat tissue, ferrocene then attains (1 or 2 days after oral administration) concentrations in the liver which are higher than those in the fat.

Use of isolated perfused liver preparations obviates the problems inherent in the *in vivo* situation (viz.: the relative distribution patterns of the compounds being examined) and permits study on a more controlled level. Indeed, as can be seen from the *in vitro* data, once the effects of distribution are removed, FIPA exerts its competitive inhibition at concentrations considerably lower than those of the substrate isopropylamphetamine.

The results *in vivo* and *in vitro* substantiate each other, and indicate that, although FIPA has considerable differences in structure from the arylalkylamines that are normally metabolized by the hepatic microsomal enzymes, it nevertheless demonstrates a considerable affinity for these enzymes. This is another example of the less rigid structural specificity of drug-metabolizing enzymes, when compared with pharmacological receptors.

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